International Journal of Engineering Science Invention
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org Volume 2 Issue 11‖ November 2013 ‖ PP.14-19

Nutritional Studies on Rats Fed Diets Formulated From Treated and Raw Samples of Jatropha Curcas Seed

BY

[1,]Nwala , [2,]C. O.Akaninwor, J. O. And [3,]Monanu, M. O.
[1,2,3],*Department ofBiochemistry, University of Port Harcourt, Choba, Port Harcourt, Nigeria*

ABSTRACT :*The nutritional qualities of untreated and treated sample of J.curcas meals were evaluated using wistar albino rats. the study revealed that the food intake was higher in the diets formulated from treated samples and control than the one from untreated samples. the growth rate (GR), protein efficiency ratio (PER) and food transformation index (FTI) of the rats were significantly ($p<0.05$)) better in diets 3, 4, 5 and 7(control diet), than in diets 1, 2 and 6. The disparity in the results obtained from these diets was considered to be an outcome of the levels of anti-nutrients still present in some of the diets. This implies that irrespective of the high protein content in J.curcas seeds, the treatments/processing of the samples in this investigation that drastically reduced the anti-nutrients: phorbol esters and trypsin inhibitors, enhanced the nutritional quality of the seeds and hence had no negative impact on the experimental animals.*

KEYWORD: *Food transformation index, food intake, lectin, phorbolesters, protein efficiency ratio.*

I. INTRODUCTION

Jatropha Curcas, commonly known as physic nut or purging nut belongs to the Euphorbiaceae family. It is a multi-purpose tree and of significant economic importance. It is well adapted to arid and semi-arid conditions and often used for prevention of soil erosion [1]. The seed of physic nut are good sources of oil, which can be used as a substitute for diesel. They are used also in medicines, soap and cosmetic manufacture in various tropical countries [2]. Although the seed meal, after extraction of oil, is rich in protein, it is toxic to rat, mice and ruminants and therefore cannot be used as animal feed. Several cases of J.curcas nut poisonings in humans after accidental consumption of the seed have been reported with symptoms of giddiness, vomiting and diarrhea [3]. The meal has high trypsin inhibitor and lectin activities, which could be inactivated by heat treatment. In addition, high concentration of the anti-metabolic, metal-chelating and heat-stable factor, phytic acid, has been reported in jatrophameal [4]. Apart from these, phorbol esters that are present at high levels in the kernels have been identified as the main toxic agent responsible for toxicity [5]. The defatted meal has been found to contain high amounts of protein, which ranged between 50% and 62%. Though various processing techniques have been attempted, no treatment has been successful in completely eliminating the anti-metabolic factor and toxic principles of defatted jatropha meal [2]. The J.curcas plant has high agro-industrial potential because of its various beneficial products. The oil extracted from the seeds can be used as a substitute for diesel after trans-esterification. The residual protein rich seed cake, remaining after extraction of the oil could be a protein-rich ingredient in feeds for poultry, pigs and cattle if it could be detoxified.The present research wastherefore designed to study the nutritional quality of the meal from seeds through feeding trials with rat and the effect of various treatments (hydrothermal processing, solvent extraction, solvent extraction and treatment with $NaHCO_3$) to inactivate the anti-nutritional factors in defatted jatropha meal.

II. MATERIALS AND METHODS

2.1PROCESSING OF J. CURCAS SAMPLES

The seeds of the*J.curcas* plant obtained from Igbo in Etche local Government Area of Rivers state, Nigeria, were de-hulled to gain access to the endocarp, which is the sample material. These were sun-dried and blended to powdery form with a high speed blender.The seed samples were then stored in an air-tight polythene bag and kept in a refrigerator at 4^0C prior to analysis.

2.2TREATMENT OF SAMPLES

The treatment of *Jatropha curcas* samples were carried out as described by Martinez-Herrera *et al*., [2].

2.2.1DE-FATTING OF SAMPLES

About 300-500g of each ground sample was de-fatted by extracting the oil in a soxhlet type extractor using petroleum ether (40-60^{o}C). After taking the de-fatted samples out of the soxhlet apparatus, they were evenly spread on a tray lined with aluminium foil. The tray was kept overnight in a fume cupboard to rid off any remaining petroleum ether and also for the defatted samples to be dry.

2.2.2TREATMENT 1

A portion of the *Jatropha curcas* de-fatted sample was autoclaved at 121^{o}C 60 minutes at 66% moisture and then lyophilized.

2.2.3TREATEMENT 2

Another portion of the de-fatted sample was treated with 0.07% $NaHCO_3$ solution in the ratio of 1:5 (W/V) and immediately autoclaved at 121^{o}C for 20 minutes. The autoclaved sample was freeze-dried as it was (without removing any supernatant).

2.2.4TREATEMENT 3

Another portion of the de-fatted sample was extracted with 90% ethanol for 2 hours at room temperature with constant stirring. The sample to solvent ratio was 1:10 (W/V). The solvent was removed by filtration and the residue was freeze-dried.

2.2.5 TREATMENT 4

The fourth portion of the de-fatted sample, after undergoing treatment similar to that of treatment 3 above, was air-dried, mixed with 0.07% $NaHCO_3$ solution in the ratio of 1:5 (W/V) and subjected to autoclaving at 121^{o}C for 20 minutes. After removing the residual water by freeze-drying, the sample was ready for use.

2.3 DIET FORMULATION

Seven (7) diets were formulated using standard rat feed, corn starch, jatropha seed, leaf and stem-bark samples (raw and treated), palm-oil, non-nutritive cellulose, vitamin and mineral mixtures. Diet (1) contained raw jatropha samples. Diet (2) contained raw jatropha samples autoclaved at 121^{o}C (15 psi) for 60 minutes; diet (3) jatropha samples treated with 0.07% $NaHCO_3$solution and autoclaved at 121^{o}C (15 psi) for 20 minutes, diet (4) jatropha samples extracted with 90% ethanol, diet (5), jatropha samples extracted with 90% ethanol, mixed with 0.07% $NaHCO_3$ and autoclaved at 121^{o}C (15 psi) for 20 minutes. The sixth diet (6) was the basal diet (protein free diet) while diet (7) was made from the standard rat feed and served as the control diet. The diets were formulated to provide thirteen percent (13%) protein in the diet and the protein sources were added at the expense of the corn starch. See tables 1, 2 and 3 below:

2.4PROXIMATE ANALYSIS

The moisture, crude fat (lipid), ash, crude fibre, crude protein and carbohydrate contents in the raw *J. curcas* sample were determined Association of Official Analytical Chemists [6].

2.5 ANTI-NUTRIENTS CONTENT EVALUATION

2.5.1 DETERMINATION OF PHYTATE CONTENT

The phytic acid contents of samples were determined by a colorimetric procedure as described by Makkarand Becker[7].

2.5.2 LECTIN CONTENT ESTIMATION

The lectin content of the samples was estimated using haemagglutination assay as reported by Aregheore*et al.*,[8].

2.5.3 DETERMINATION OF TRYPSIN INHIBITOR CONTENT

The trypsin inhibitors' activities of the samples were determined as outlined by Gaborit*etal.*, [9].

2.5.4 DETERMINATION OF PHORBOL ESTERS CONTENT

The phorbol esters estimation of the sample was done as describedby Aderibigbe*et al.*,[10]. Phorbol-12-myristete-13-acetate was used as the standard during the determination of phorbol ester concentration.

2.6 ANIMAL FEEDING EXPERIMENT

The method of Aregheore*et al.*, [11] was adopted.

2.7 STATISTICAL ANALYSIS

The data were analyzed using tables, range, means, percentages, standard deviation and hence standard error (SE). Also, all the data obtained were subjected to analysis of variance (ANOVA) using computer aided science planning and scheduling system (SPSS) compared using Duncan's multiple range test [12] at 5% level of significance.

III. RESULT AND DISCUSSION

The results are presented in tables 1 – 7below:

TABLE 1: PROXIMATE COMPOSITION OF RAW SAMPLES OF JATROPHA CURCAS SEED

% COMPOSITION	SEED
Moisture	4.30 ± 0.17^{a}
Lipid	40.90 ± 0.23^{a}
Ash	4.00 ± 0.15^{a}
Fibre	17.40 ± 0.17^{a}
Protein	27.13 ± 0.32^{a}
Carbohydrate	6.28 ± 0.16^{a}

Values are means±standard deviation of triplicate determinations.
Means in the same row with different superscript letters are significantly different at the 0.05 level.

TABLE 2: PROXIMATE COMPOSITION OF TREATED SAMPLES OF JATROPHA CURCAS (SEED).

% COMPOSITION	TREATMENT 1	TREATMENT 2	TREATMENT 3	TREATMENT 4
Moisture	1.80 ± 0.29^{a}	2.00 ± 0.46^{a}	1.87 ± 0.39^{a}	1.95 ± 0.55^{a}
Lipid	0.10 ± 0.00^{a}	0.20 ± 0.06^{a}	0.10 ± 0.03^{a}	0.15 ± 0.00^{a}
Ash	6.30 ± 0.26^{a}	6.10 ± 0.32^{a}	5.97 ± 0.52^{a}	6.20 ± 0.14^{a}
Fibre	51.75 ± 0.43^{a}	50.77 ± 0.16^{ab}	49.63 ± 0.36^{b}	50.49 ± 0.33^{ab}
Protein	37.19 ± 0.11^{a}	38.34 ± 0.20^{ab}	40.08 ± 0.58^{b}	39.32 ± 0.92^{ab}
Carbohydrate	2.86 ± 0.5^{a}	2.59 ± 0.34^{a}	2.35 ± 0.23^{a}	2.20 ± 0.12^{a}

Values are means±standard deviation of triplicate determinations.
Means in the same row with different superscript letters were significantly different at the 0.05 level.
Treatment 1: Heat treated (autoclaved at 121^{o}C for 60 mins).
Treatment 2: Treated with 0.07% $NaHCO_3$ and autoclaved at 121^{o}C for 20 mins).
Treatment 3:Extracted with 90% Ethanol.
Treatment 4:Extracted with 90% Ethanol and mixed with 0.07% $NaHCO_3$ and autoclaved at 121^{o}C for 20 mins).

TABLE 3: COMPOSITION OF DIETS FORMULATED FROM RAW AND TREATED SAMPLES OF JATROPHA CURCAS SEED

INGREDIENTS	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	DIET 6	DIET 7
Standard rat feed	33.00	33.00	33.00	33.00	33.00	-	65.00
Jatropha meal	48.00	35.00	34.00	32.00	33.00	-	-
Corn flour starch	6.00	19.00	20.00	22.00	21.00	87.00	22.00
Red palm oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Standard vitamin mixture	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Standard mineral mixture	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Non-nutritive cellulose	400	4.00	4.00	4.00	4.00	4.00	4.00
Total in gram	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Diet 1 = Raw jatropha seed
Diet 2 = 60 minutes autoclaved (Heat treatment only)
Diet 3 = 0.07% $NaHCO_3$ solution treated and autoclaved for 20 minutes
Diet 4 = Extracted with 90% ethanol
Diet 5 = Extracted with 90% ethanol, treated with 0.07% $NaHCO_3$ and autoclaved for 20 minutes
Diet 6 = Basal diet
Diet 7 = Control diet (prepared from standard rat feed).

TABLE 4: PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS FORMULATED FROM SAMPLES OF JATROPHA CURCAS SEED.

% COMPOSITION	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	DIET 6	DIET 7
Moisture	8.00 ± 0.46^{a}	7.66 ± 0.38^{a}	8.10 ± 0.06^{a}	6.77 ± 0.44^{a}	7.55 ± 0.32^{a}	7.43 ± 0.25^{a}	8.03± 0.22^{a}
Lipid	16.73 ± 0.42^{a}	6.31 ± 0.18^{bcdefg}	5.87 ± 0.50^{bcdefg}	5.45 ± 0.26^{bcdef}	6.05 ± 0.40^{bcdefg}	6.19 ± 0.11^{bcdefg}	7.32± 0.18^{bcefg}
Ash	5.78 ± 0.45^{a}	5.49 ± 0.28^{a}	6.23 ± 0.13^{a}	6.38 ± 0.22^{a}	5.95 ± 0.54^{a}	6.50± 0.29^{a}	5.45± 0.26^{a}
Fibre	37.44 ± 0.25^{ac}	41.13 ± 0.13^{bdefg}	38.91 ± 0.52^{acde}	40.70 ± 0.40^{bcdefg}	39.89 ± 0.51^{bcdef}	41.70± 0.23^{bdefg}	42.36 ± 0.21^{bdfg}
Protein	13.55 ± 0.32^{abce}	14.34 ± 0.20^{abcdeg}	14.89 ± 0.51^{abcdeg}	15.90 ± 0.43^{bcdeg}	14.60 ± 0.35^{abcdeg}	6.67± 0.39^{f}	15.65 ± 0.29^{bcdeg}
Carbohydrate	18.50 ± 0.20^{a}	25.00 ± 0.58^{bcde}	26.00 ± 0.14^{bcdeg}	24.80 ± 0.46^{bcde}	25.96 ± 0.55^{bcdeg}	31.51± 0.29^{f}	27.19± 0.11^{ceg}

Values are means±standard deviation of triplicate determinations.
Means in the same row with different superscript letters were significantly different at the 0.05 level.
Diet 1: Prepared from raw samples of Jatropha curcas.
Diet 2: From heat treated sample (autoclaved at 121^{o}C for 60mins).
Diet 3: From sample treated with 0.07% $NaHCO_3$ and autoclaved at 121^{o}C for 20mins.
Diet 4 From sample extracted with 90% ethanol.
Diet 5: From meal extracted with 90% ethanol and mixed with 0.07% $NaHCO_3$ and autoclaved at 121^{o}C for 20mins.
Diet 6: Basal diet (protein free)
Diet 7: Formulated from standard feed (control diet)

TABLE 5: ANTI-NUTRIENT COMPOSITION OF RAW SAMPLES OF JATROPHA CURCAS SEED.

PARAMETERS	SEED
Phytate (%)	0.40 ± 0.09^{a}
Lectin (mg/ml)	10.15 ± 0.20^{a}
Trypsin inhibitor (mg/100g)	3164.93 ± 0.47^{a}
Phorbol esters (mg/100g)	318.13 ± 0.31^{a}

Values are means±standard deviation of triplicate determinations.
Means in the same row with different superscript letters were significantly different at the 0.05 level.

TABLE 6: EFFECTS OF VARIOUS TREATMENTS ON ANTI-NUTRIENT IN JATROPHA CURCAS SEED

PARAMETERS	TREATMENT 1	TREATMENT 2	TREATMENT 3	TREATMENT 4
Phytate (%)	0.29 ± 0.11^{a}	0.28 ± 0.09^{a}	0.32± 0.14^{a}	0.35 ± 0.16^{a}
Lectin (mg/ml)	1.80 ± 0.20^{acd}	4.80 ± 0.52^{b}	1.97± 0.46^{c}	2.13 ± 0.35^{acd}
Trypsin inhibitor (mg/100g)	59.00 ± 0.57^{a}	78.00 ± 0.28^{b}	175.00 ± 0.35^{c}	67.00 ± 0.46^{d}
Phorbol esters (mg/100g)	183.40 ± 0.36^{a}	56.00 ± 0.23^{b}	16.00± 0.00^{c}	8.00 ± 0.58^{d}

Values are means±standard deviation of triplicate determinations.
Means in the same row with different superscript letters were significantly different at the 0.05 level.

TABLE 7: FOOD INTAKE, GROWTH RATE, PROTEIN EFFICIENCY RATIO (PER), FOOD TRANSFORMATION INDEX (FTI) OF RATS FED WITH MEAL FORMULATED FROM SAMPLES OF JATROPHA CURCAS SEED.

PARAMETERS	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	DIET 6	DIET 7
Daily Food Intake (g)	3.21± 0.29^{a}	5.10± 0.38^{b}	7.17± 0.29^{c}	12.43± 0.25^{dg}	10.07± 0.21^{ef}	9.87± 0.50^{ef}	13.79± 0.46^{dg}
Initial average weight (g)	99.00± 0.29^{a}	85.00± 0.52^{b}	90.00± 0.40^{cg}	92.00± 0.26^{d}	95.00± 0.46^{e}	80.00± 0.35^{a}	89.00± 0.12^{cg}
Final average weight (g)	70.00± 0.58^{a}	84.00± 0.35^{b}	95.00± 0.87^{c}	102.00± 0.12^{deg}	101.00± 0.29^{de}	73.00± 0.00^{f}	104.00± 0.40^{dg}
Body weight gain/loss (g)	-29.00± 0.20^{a}	-1.00± 0.00^{b}	5.00± 0.46^{ce}	10.33± 0.44^{d}	6.00± 0.32^{ce}	-7.00± 0.58^{f}	15.00± 0.52^{g}
Daily weight gain (g)	-2.07± 0.14^{a}	-0.07± 0.00^{bcdefg}	0.36± 0.06^{bcdefg}	0.71± 0. 32^{bcdefg}	0.42± 0.16^{bcdefg}	-0.50± 0.20^{bcdef}	1.07± 0.46^{bcdeg}
Protein Efficiency Ratio (PER)	-51.75± 0.43^{a}	-1.00± 0.17^{b}	3.27± 0.16^{cdeg}	3.55± 0.32^{cdeg}	2.87± 0.50^{cde}	-7.14± 0.25^{f}	4.86±0.50^{dg}
Food Transformation Index (FTI)	-1.55± 0.31^{a}	-72.86± 0.50^{b}	19.92± 0.52^{c}	17.51± 0.29^{d}	23.44± 0.24^{e}	-19.74± 0.43^{f}	12.89± 0.51^{g}

Values are means±standard deviation of triplicate determinations.
Means in the same row with different superscript letters were significantly different at the 0.05 level.

The various treatments methods adopted in this study reduced to the barest tolerable levels, the content of the various anti-nutrients in the sample .Trypsin inhibitors and phorbol esters which are the major anti-nutrients in *J. curcas* seed were reduced to about 98.13% and 97.49% respectively.The daily food intake of rats fed with diets formulated from the seeds of *J.curcas*for the 14-day experimental period was highest in rats fed the control diet(diet 7).It has been reported that in rat, food intake is influence by a variety of factors such as the (i) amino acid pattern of the dietary protein (ii)taste (iii)smell and (iv)texture of the diet[11]. The low food intake of rat in diet 1 and 2 could be attributed to probably such factors as taste, smell and texture but not to the amino acid pattern of the *J. curcas*meal because work done by Makkar and Becker [5], revealed a good balance of amino acid but for lysine and the sulphur amino acids. The rat fed diets 1,2 and 6 had weight losses while those fed diet 3,4,5 and 7 had weight gains. The weight loss in diet 6(basal diet) may be due to the low protein level and this was supported by works done by Bender [13] and Aregheore*et al*.,[11], who reported weight losses in rats fed low protein diets. For diets 1 (formulated from raw seed) and 2 (moist heat treatment), the negative weight change may be attributed to the presence of the anti-nutritional factors prominent among which were phorbol esters and trypsin inhibitors known to interfere with protein metabolism and food acceptance. A similar observation was made in which the presence of trypsin inhibitor activity and phorbol esters in uncooked food caused diminished growth in rats, chicken and other experimental animals by various authors [14], [15]. Other experimental diet showed weight increases in the rat with the control diet (diet7) having the highest value. This showed that the control diet was superior to the other diet. However,the fact that the other experimental diets were able to support growth in the rats suggested that the levels of the residual anti-nutrients did not interfere with protein metabolism. Diets 1,2and 6 had negative values of PER. This observation were similar to the to the work of Makkar and Becker [7] who reported negative PER values for rats fed raw African locust bean and Ayalogu*etal*., [16] who reported weight losses and negative PER for rats fed basal diet. Conversely, rats fed diets 3,4,5 and 7 control diet (diet7) had positive values of PER. These diets may be said to be comparable to the control diet, since they also supported the growth of the experimental animals, though not at equal proportions.The food Transformation index (FTI) of the experimental animals for the various diets followed the same trend as the PER.

IV. CONCLUSION

Finally, the results obtained from this study showed that jatropha meal protein is of high biological value, having being able to support growth in the experimental animals. The rats fed diet formulated from the untreated seed sample had weight loss, while the ones fed diet formulated from treated seed sample had weight gain, implying that proper treatment of jatropha seed meal had great impact on its nutritional quality.

REFERENCCES

[1] J. Heller. Physic nut (J. curcas L.). Promoting the conservation and use of und-utilized and neglected crops. I. Gatersleben: Institute of Plant Genetics and Crop Plant Research/Rome: Inernational Plant Genetic Resources Institute, 1996.

[2] J. Martinez-Herrera, P. Siddhuraju, G. Franci, G. Davila-Ortiz, K. Becker. Chemical Composition, Toxic/Antimetabolic Constituents, and Effects of Different Treatments on their levels, in four Provenances of Jatropha Curcas L. from Mexico.Food Chemistry.96, 2006, 80-89.

[3] K. Becker, and H. P. S. Makkar. Effects of Phorbilesters in Carp (Cyprinuscarpio L.).Veterinary Human Toxicology.40, 1998, 82-86.

[4] H. P. S. Makkar, K. Becker and B. Schmook.Edible Provenance of Jatropha curcas from Quintana Roo State of Mexico and Effect of Roasting on Anti-nutrient and Toxic Factors in Seeds.PlantFoods for Human Nutrition.52, 1998, 31-36.

[5] H. P. S. Makkarand K. Becker. Jatropha Curcas toxicity: Identification of toxic principles. In: 5th Inter. Symposium on poisonous plants.Sara Angela, Texas USA. 1997.

[6] Association of Official Analytical Chemists (AOAC). Official Methods of Analysis, 15thEdn.Washington DC, USA, 1990.

[7] H. P. S. Makkar and K. Becker. Nutritional Studies on Rats and Fish (Carp Cyprinuscarpio) Fed Diets Containing Unheated and Heated Jatropha curcas Meal of a Non-toxic Provenance. Plant Foods for Human Nutrition.53, 1999, 183-192.

[8] G. M. Aregheore. H. P. S. Makkar and K. Becker, K. Assessment of Lectin Activity in a Toxic and Non-Toxic Variety of Jatropha curcas Using Latex Agglutination and Haemagglutination Methods and Inactivation of Lectin by Heat Treatment. J. Sci Food Agric.77, 1998,349-352.

[9] T. Gaborit, L. Quillien, and J. Gueguen.Determination of Trypsin Inhibitor Activity in Seeds by Microtitre plate method. In: Recent Advances of Research of Anti-nutritional Factors in Legume Seeds. Wageningen, The Netherlands. 1993.

[10] O. A. Aderibigbe, C. O. L. E. Johnson, H. P. S. Makkar, K. Becker, and L. Foidl. Chemical Composition and Effect of Heat on Organic Matter and Nitrogen-Degradability and Some Anti-nutritional Components of Jatropha Meal.J. Ani. Feed Sci. Technol.67, 1997, 223-243.

[11] E. M. Aregheore, K. Becker and H. P. S. Makkar.Detoxification of a Toxic Variety of Jatropha curcas Using Heat and Chemical Treatments and Preliminary Nutritional Evaluation with Rats. S.pac. J. Nat. Sci.21, 2003, 50-56.

[12] D. B. Duncan. Multiple Range and Multiple F. Tests, Biometrics.11, 1955, 1-42.

[13] A. E. Bender.Evaluation of Protein Quality Methodological Consideration Proc. Nutri.Soc.1983, 41267-275.

[14] S. Mole, J. C. Rogler, C. J. Morrel and L. G. Butler.Histologicalalterations to the pancrease and the intestinal tract produced by rawfebabean Viciafaba l minor) diets in growingchicks. British PoultrySci. 30, 1990, 101 – 114.

[15] N. N. Pathak. andS. K. Ranjahan. Nutritional Studies with Salseed-meal as a component of fisher ratios in the large white Yorkshire Pigs. Int. J. of Animal Sci. 43, 1977, 424-427.

[16] E. O. Ayalogu, E. N. Onyeike and C. C. Okaraonye.Evaluation of the Nutritional Status of Rats Fed Diets Formulated from Larvae of Raphia Palm Bettle (Oryctespheonicis).Nig. J. Biochem and Mol. Biol. 18 (1), 2003, 47-51.